

## A simple method based on laboratory inoculum and field inoculum for evaluating potato resistance to black scurf caused by *Rhizoctonia solani*

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A two-step method was developed to evaluate potato resistance to black scurf caused by *Rhizoctonia solani*. Tuber piece inoculum was first conducted in the laboratory, which was also first reported in this study. After inoculation with pathogen discs and culture for 48 h, the necrotic spots on the inoculated potato pieces were generated and measured by the crossing method. Further evaluation was conducted through field experiments using a wheat bran inoculum method. The wheat bran inoculum was placed into the pit dispersedly and surrounded seed tubers. Each cultivar or line was subjected to five treatments of 0-, 2-, 3-, 4-, and 5-g soil inoculum. The results showed that 2–4 g of wheat bran inoculum was the optimum for identifying tuber black scurf resistance. The laboratory scores positively correlated with the incidence and severity of black scurf in the field. According to the results in the laboratory, relatively resistant cultivars could be selected for further estimation of tuber black scurf resistance in field experiments. It is a practical and effective screening method for rapid identification of resistant potato germplasm, which can reduce workload in the field, shorten time required for identification.

**Key Words:** potato, *Rhizoctonia solani*, black scurf, wheat bran inoculum, tuber piece inoculum, two-step method.

### Introduction

*Rhizoctonia solani* Kühn is a common and commercially important pathogen that infects all below-ground portions of the potato plant and causes lesions and decay on potato seeds, sprouts, roots, stolons and stems, which lead to yield losses. The brown to black sclerotia on the surfaces of mature tubers are referred to as black scurf (Atkinson *et al.* 2010, Banville 1989, Carling *et al.* 1989, Simons and Gilligan 1997a, Woodhall *et al.* 2007, 2008). The loss of quality caused by black scurf, particularly for fresh market potatoes, financial loss is the most economically damaging aspect of this disease (Atkinson *et al.* 2010, Keiser *et al.* 2012). According to described by Keiser (2008), yield losses caused by black scurf reached 50%, severely affecting potato production and resulting in marked economic losses for farmers.

Black scurf has been found in all potato production areas of the world (Bakali and Martin 2006). In recent years, with an increase in potato planting area and continuous cropping, soil-borne diseases, including potato black scurf caused by *R. solani*, have become increasingly serious. As one of the largest potato-producing countries, China has also been

widely affected by species of *Rhizoctonia*, especially in some major potato-producing areas such as Inner Mongolia (Qu *et al.* 2008). We investigated the occurrence of black scurf on tubers in a heavily infected field in Wulanchabu city of Inner Mongolia in 2008, and found a morbidity of up to 100% caused by black scurf (Zhang *et al.* 2012).

Conventional control measures for *R. solani* are chemical (Bautista *et al.* 2007, Grosch *et al.* 2005, Lahlali and Hijri 2010). However, they have little effect and may cause environmental pollution (Brewer and Larkin 2005, Jiang *et al.* 2005, Kurzawińska and Mazur 2008). In addition, black scurf development on daughter tubers can be minimized by harvesting quickly after vine desiccation, but it can not control completely. Crop rotation with non-susceptible crops for *Rhizoctonia* for 3–5 years is helpful to reduce both the incidence and severity of this disease. However, rotation is difficult to conduct in the main planting area of potato (Bakali and Martin 2006).

Accordingly, the selection and cultivation of resistant cultivars has become one of the most economical and effective way to control tuber black scurf (Naz *et al.* 2008). However, few resistant cultivars are available to date (Bains *et al.* 2002, Djébali and Belhassen 2010, Khandaker *et al.* 2011, Leach and Webb 1993, Naz *et al.* 2008, Olanya *et al.* 2009, Scholte 1989, Yanar *et al.* 2005). Mikheeva (1988) studied with some potato cultivars for field resistant to *R. solani* and found that the Erfolg cultivar was highly resistant to *R. solani*, which showed almost no infection of the

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tubers and roots. Pietkiewicz and Chorożewski (1983) evaluated 44 varieties of potato and found that seven cultivars were resistant to *R. solani*, of which three cultivars, Odra, Poprad and Reda were highly resistant. Khandaker *et al.* (2011) screened 25 potato germplasms to investigate the resistance level of the recommended varieties and lines as well as true potato seeds against *R. solani*, however no germplasm was found resistant to *R. solani*, only six germplasms appeared to be moderately resistant.

Traditionally, resistance to fungal diseases such as *Rhizoctonia* black scurf, have been scarcely considered in any potato breeding program, because of the extremely wide host range of the pathogen (Bakali and Martín 2006, Ogoshi 1987, Woodhall *et al.* 2007) and the saprophytic life cycle (Grosch *et al.* 2005). Some reports found the differences in disease expression of cultivars and breeding lines, which has encouraged the screening for varietal differences in susceptibility (Bains *et al.* 2002, Djébali and Belhassen 2010, Leach and Webb 1993, Naz *et al.* 2008, Olanya *et al.* 2009, Scholte 1989, Yanar *et al.* 2005). Therefore, it is essential to develop effective resistance appraisal techniques for accurately screening potato germplasm. To date, there are few reports on the resistant breeding of potato to black scurf and the identification and screening of resistant potato cultivars or lines, considering that there is no standard inoculation method and system of disease classification (Bains *et al.* 2002, Djébali and Belhassen 2010, Naz *et al.* 2008, Secor and Gudmestad 1999). These deficiencies call for the development of systematic identification method for evaluating and screening potato germplasm resistant to *R. solani*.

The objective of this study is to: (1) explore a new identification method with potato pieces inoculated in laboratory; (2) ascertain the influence of inoculum levels and determine inoculation method in field; (3) evaluate the resistance of 20 potato cultivars or lines against *R. solani*; (4) and propose a two-step method to evaluate resistance to *R. solani* and rapidly screening resistant potato cultivars.

## Materials and Methods

### Potato materials

For resistance evaluation, 20 potato materials were used, including 14 commercial cultivars (Longshu 3, Longshu 6, Longshu 7, Desiree, Shuixihong, Sepihong, Atlantic, Zihuabai, LK99, Kexin 1, Shepody, Heimeiren, Favorita, and Qingshu 168) widely planted in China and 6 potato lines obtained from Hokkaido University, Japan (J08-1, J08-4, J07-2, J07-5, J07-6, and J07-7). These cultivars or lines were all virus-free and were maintained at the Research Center of Potato Breeding of Inner Mongolia Agricultural University.

### Pathogen

As a destructive soil-borne pathogen, *R. solani* can cause diseases in many plants world-wide. Currently, according to the ability to anastomose with tester isolates belonging to

established AGs (Anastomosis Groups), isolates of the fungus can be classified into different groups (Bakali and Martín 2006). *Rhizoctonia solani* AG2-1 is the main and dominant group in China, which has representative and strong pathogenicity. In the present study, isolate WC-16 (available from Zhuo Yu) of *R. solani* AG2-1 was used as the pathogen of potato black scurf. It was isolated from infected potato Favorita from a field in Wuchuan region (China) and tested highly pathogenic in Favorita potato tubers. The pathogen was stored in potato sucrose agar (PSA, 2% sucrose, 1.2% agar) at 4°C in the Plant Pathology Laboratory of Inner Mongolia Agricultural University.

### Tuber piece inoculum assay in the laboratory

Healthy potato tubers of almost the same maturity and size were used as inoculation materials. These tubers were surface sterilized for 20 min with 0.5% KMnO<sub>4</sub> solution. Under aseptic conditions, 0.6 cm thick pieces were cut from middle of the tubers in view of the non-uniform distribution of nutrients in potato tubers. These tuber pieces placed into 90 mm culture dishes with two layers of sterilized filter paper containing 10 mL sterile distilled water (approximately 88% relative humidity). Pathogen discs of 0.5 cm diameter cultured for 4 days in PSA at 25°C under dark conditions were placed at the centers of the potato pieces with the hyphal side down. Each potato piece was inoculated with only one disc. The inoculated potato pieces were cultured in the dark at 25°C for 48 h. The diameters of the necrotic spots were measured by the crossing method, and the area of the spots was calculated as a measure of tuber resistance. The tuber piece inoculation was performed in April 2009 and 2010 with 10 replications. The agar, KMnO<sub>4</sub> and sucrose were derived from Sinopharm Chemical Reagent Co., Ltd. in Shanghai of China.

### Fungal preparation for soil inoculums

Wheat bran (100 g) and distilled water (200 mL) were mixed well in a 500 mL conical flask, autoclaved at 121°C for 40 min. Each flask was inoculated with 10 discs (0.8 cm diameter) containing the pathogen cultured for 4 days in PSA. These were cultured at 25°C under dark light condition. After 30 days, the wheat bran with hyphae and a small amount of sclerotia was collected, and naturally dried. Most of the wheat bran culture became very soft and fragile, and was grounded into powder by hands. And a small part of culture is still very hard, which is grinded with mortar and pestle lightly.

### Field trials

Field experiments were conducted during the 2009 and 2010 growing seasons. The 20 potato cultivars or lines were planted in a screen house in the Horticulture Science and Technology Demonstration Area of Hohhot City in May 2009, in light sandy soil, containing 18.5 g/kg organic matter, 74 mg/kg hydrolysis nitrogen, 23.0 mg/kg available phosphorus, and 116 mg/kg rapidly available potassium (Detected

by the Testing Center of Agricultural Products Quality and Safety in Inner Mongolia). Each cultivar or line was subjected to five treatments at 0-, 2-, 3-, 4-, and 5-g of wheat bran inoculum, with the 0-g treatment as a control. The seed tubers were sterilized for 20 min with 0.5% KMnO<sub>4</sub>, then washed out and cut into 30–50 g seed tuber for plant. The wheat bran inoculum was placed into the pit dispersedly and surrounded seed tubers. Each seed tuber was inoculated with one inoculum dose of the wheat bran containing hyphae and sclerotinia. All the 20 potato materials were planted in one plot randomly per treatment, and each cultivar or line was planted in two rows. Each row contained 20 plants with 30-cm spacing within plants and 60-cm spacing between rows. The trial designed three replications per treatment. During the growth period of 133 days, the potato entries were not fertilized and were irrigated three times.

In May 2010, an experimental field was established on the Inner Mongolia Agricultural University farm, in an area of higher soil fertility than that in 2009. The soil fertility was as follows: the organic matter was 18.7 g/kg, hydrolysis nitrogen was 75 mg/kg, available phosphorus was 24.50 mg/kg, and rapidly available potassium was 118 mg/kg. The fungal inoculum was 2 g per seed tuber, with 0-g inoculum used as a control. The experimental design, field inoculation method, and management measures were as in 2009.

#### *Disease severity of black scurf*

After harvest in September 2009 and 2010, tubers were kept in a dry place with room temperature (15 to 18°C) for 2 days to dry the sclerotia and then washed carefully to remove soil. The tubers with the weight more than 20 g were evaluated for disease severity, while those tubers which were immature, infected by other pathogen, damaged by pest, or had the weight less than 20 g were removed. Each replication of each treatment randomly selected 100 tubers to evaluate the disease severity of black scurf. The ratio of tuber surface area covered with sclerotia was used as a general method to evaluate potato black scurf. The tuber surface area covered with sclerotia was estimated using the following scale:

- 0: no sclerotia present
- 1: less than 1% of tuber area covered
- 2: 2%–10% of tuber area covered
- 3: 11%–20% of tuber area covered
- 4: 21%–50% of tuber area covered
- 5: 51% or more of tuber area covered

Disease index (DI) and relative resistance index (RRI) were calculated by the following formulas.

$$DI = \frac{(n_0 \times 0) + (n_1 \times 1) + (n_2 \times 2) + (n_3 \times 3) + (n_4 \times 4) + (n_5 \times 5)}{Y \times 5} \times 100$$

where  $n_x$  = number of tubers in severity class  $x$  and  $Y$  = total number of tubers

$$RRI = 1 - \frac{DI_x}{DI_{max}}$$

where  $DI_x$  = disease index of the observed tuber and  $DI_{max}$  = the maximum disease index of all cultivars or lines in the same treatment.

Black scurf resistance was measured with the relative resistance index (RRI) as follows:

- 1: immune
- 0.99–0.80: highly resistant
- 0.79–0.60: moderately resistant
- 0.59–0.40: moderately susceptible
- 0.39–0.00: highly susceptible

#### *Statistical analysis*

Analysis of variance (ANOVA) was performed by the general linear model (GLM) procedure of SAS 9.1 (Cary, NC, USA).

## **Results**

#### *Response to R. solani on tubers by tuber piece inoculation*

Resistance of tubers to *R. solani* was identified by tuber piece inoculation in the laboratory during 2009 and 2010. Hyphae on tuber pieces began to grow and expand 4 h after inoculation. Potato tissue became brown and necrotic, and typical necrotic spots and a clearly expanding colony rim appeared 8 h after inoculation. At 48 h after inoculation, the lesion areas showed significant differences among cultivars or lines ( $F = 6.62$ ,  $P < 0.01$ ). Table 1 showed the lesion area of all 20 materials. ‘Desiree’ had the minimum lesion area, with an average of 0.53 cm<sup>2</sup>, while ‘Atlantic’, ‘Shepody’, and some Japanese lines showed large lesion areas.

#### *Evaluation of tuber black scurf resistance in field*

Table 2 showed the evaluation of tuber black scurf resistance of the 20 potato cultivars or lines with different wheat bran inoculum levels in 2009. Wheat bran produced a large number of hyphae and sclerotia, which effectively induced on potato tubers typical symptoms of black scurf caused by *R. solani*. The control materials (with 0-g wheat bran inoculum) were not or lightly infected, with disease index lower than 14, and resistant (R) and susceptible (S) reactions could not be distinguished. Cultivar resistance was distinguished only if the disease index of the most susceptible cultivar among the measured materials should not be less than 40.0 (Shi *et al.* 2000). The potato cultivars or lines (with 2-, 3-, 4-, or 5-g wheat bran inoculum) were obviously infected, and the disease indices showed significant differences ( $p < 0.01$ ) (Table 3). The resistance scores for 2-, 3-, and 4-g inoculum levels showed generally consistent proportions of resistant and susceptible. However, when the inoculum was 5 g the proportions of resistant and susceptible cultivars reached 7.1% and 92.9%, respectively.

The ANOVA of average disease index of the three inoculum levels in 2009 and disease index in 2010 are shown

**Table 1.** Lesion area on potato pieces inoculated with *R. solani* at 48 h

Cultivars/ lines	Lesion area in 2009 (cm <sup>2</sup> )											Average	Lesion area in 2010 (cm <sup>2</sup> )											Average	Average
	1	2	3	4	5	6	7	8	9	10	1		2	3	4	5	6	7	8	9	10	Average			
Desiree	0.49	0.64	0.48	0.81	0.48	0.49	0.72	0.64	0.64	0.48	0.59		0.49	0.56	0.42	0.49	0.42	0.42	0.42	0.49	0.49	0.47	0.53		
LK99	0.63	0.64	0.48	0.63	0.63	0.56	0.63	0.36	0.48	0.49	0.55		1.44	0.64	0.72	0.63	0.63	1.21	0.81	0.64	0.64	0.72	0.81	0.68	
Zihuabai	0.64	0.72	0.72	0.72	0.72	0.56	0.49	0.72	0.72	0.64	0.67		0.64	0.72	0.72	0.81	0.81	0.72	0.64	1.1	0.72	0.81	0.77	0.72	
Kexin 1	1.1	1.69	0.9	0.9	1	0.9	0.81	1.44	1.32	0.9	1.10		0.81	0.9	0.36	0.42	0.72	0.9	0.81	0.9	0.64	0.49	0.70	0.9	
Qingshu 168	0.9	0.81	0.9	1	1	0.9	0.9	0.81	0.9	0.9	0.90		1	0.9	1	1	1	0.9	1	1.1	1.1	1	1.00	0.95	
J08-4	1.21	1.32	0.81	1.21	1.1	0.81	0.72	1.1	0.81	1	1.01		1.82	2.1	1.96	1.56	1.44	1.44	1.32	1.69	1.32	1.44	1.61	1.31	
Longshu 7	1.21	1.1	1.44	1.44	1.56	1.44	1.32	1.1	1.32	1.1	1.30		1.82	1.56	1.56	1.56	1.56	1.96	1.69	1.69	1.82	1.82	1.70	1.5	
Longshu 3	1.56	1.56	1.56	1.44	1.21	1.44	1.44	1.56	1.44	1.56	1.48		1.56	1.69	1.56	1.69	1.44	1.44	1.56	1.56	1.82	1.69	1.60	1.54	
Heimeiren	1.56	1.44	1.44	1.56	1.56	1.56	1.44	1.56	1.44	1.44	1.50		1.69	2.1	1.44	1.44	1.56	1.82	1.69	1.69	1.56	1.82	1.68	1.59	
Longshu 6	1.44	1.56	0.56	1.69	0.56	0.81	0.49	0.81	1.32	1.32	1.06		2.56	2.4	1.69	1.96	1.82	2.56	2.56	2.4	2.72	1.96	2.26	1.66	
J08-1	1.69	3.42	3.24	3.24	1.96	1.82	1.82	1.69	1.82	1.82	2.25		1.69	1	1.69	1.44	1.56	1.21	1.69	0.81	0.56	0.81	1.25	1.75	
Favorita	1.96	1.96	1.69	1.69	1.56	1.44	1.69	1.21	1.44	1.69	1.63		1.96	1.96	1.96	2.89	1.96	1.82	1.96	1.96	2.1	1.96	2.05	1.84	
J07-5	1.82	1.69	1.82	1.69	1.69	1.82	1.96	1.96	1.96	1.69	1.81		1.96	1.82	1.96	1.96	1.96	1.96	1.82	2.1	1.96	1.82	1.93	1.87	
J07-6	1.96	1.21	0.81	0.56	1.69	1.21	0.64	0.49	0.49	1.21	1.03		2.72	1.96	2.56	4.41	2.25	3.06	2.1	2.89	4.41	1.96	2.83	1.93	
Shuixihong	1.96	2.1	2.1	1.96	2.1	2.1	1.96	1.96	1.69	1.82	1.98		1.96	2.25	2.25	1.96	2.1	1.96	2.25	2.25	1.96	2.1	2.10	2.04	
Atlantic	1.96	2.1	2.25	3.24	2.56	2.25	3.24	2.1	3.61	2.4	2.57		2.25	1.21	1.32	2.1	0.81	1.96	2.4	1.96	0.64	1	1.57	2.07	
Shepody	2.25	1.96	2.1	1.69	2.4	2.1	1.56	2.4	1.44	2.1	2.00		3.24	3.06	4	2.89	2.56	2.89	2.56	3.61	2.88	3.06	3.08	2.54	
Sepihong	2.89	3.06	3.24	3.24	3.23	3.06	3.42	3.42	3.42	3.06	3.20		3.24	3.24	3.42	3.24	3.24	3.42	3.24	3.42	3.06	3.06	3.26	3.23	
J07-7	3.61	3.8	3.06	3.61	3.61	3.42	3.24	3.24	3.06	3.06	3.37		3.6	3.6	3.6	3.8	3.6	3.6	3.61	3.61	3.06	3.06	3.51	3.44	
J07-2	3.6	3.23	3.23	3.42	3.23	3.06	3.23	2.72	3.06	3.06	3.18		6	6	5.06	4	4.84	4.2	4.41	4.2	4.84	4.2	4.78	3.98	

Correlation coefficient between average lesion area of different potato cultivars or lines inoculated with *R. solani* and the average disease index of tubers in field in 2 years,  $r = -0.455^*$ . \* indicates significant correlation; \*\* indicates extremely significant correlation.

**Table 2.** Evaluation of 20 potato cultivars or lines for tuber black scurf resistance with different soil-borne inoculum levels in 2009

Cultivars/ lines	Inoculum 0 g	Inoculum 2 g			Inoculum 3 g			Inoculum 4 g			Inoculum 5 g		
	DI	DI	RRI	Resistance evaluation	DI	RRI	Resistance evaluation	DI	RRI	Resistance evaluation	DI	RRI	Resistance evaluation
Desiree	2.8	17.18	0.82	HR <sup>a</sup>	29.52	0.66	MR	16.57	0.83	HR	30.29	0.69	MR
LK99	8.67	35.8	0.63	MR <sup>b</sup>	67.5	0.22	HS	44.74	0.55	MS	53.94	0.44	MS
Zihuabai	9.76	55.98	0.42	MS <sup>c</sup>	48.45	0.44	MS	36.79	0.63	MR	56	0.42	MS
Kexin 1	6.5	49.27	0.49	MS	40.49	0.53	MS	48.89	0.51	MS	68	0.3	HS
Qingshu 168	2.25	38.55	0.6	MR	34.23	0.61	MR	48.89	0.51	MS	49.7	0.49	MS
J08-4	1.91	50.75	0.48	MS	44.81	0.49	MS	58.85	0.41	MS	67.5	0.3	HS
Longshu 7	3.98	52.26	0.46	MS	48.44	0.44	MS	60.06	0.4	MS	51.5	0.47	MS
Longshu 3	9.15	41.6	0.57	MS	45.12	0.48	MS	49.34	0.51	MS	62.19	0.36	HS
Heimeiren	0	22.53	0.77	MR	31.08	0.64	MR	36.27	0.64	MR	50.29	0.48	MS
Longshu 6	3.15	42.35	0.56	MS	36.13	0.58	MS	33.71	0.66	MR	66.67	0.31	HS
J08-1	0	50	0.48	MS	58.1	0.33	HS	35.83	0.64	MR	22	0.77	MR
Favorita	5.3	61.33	0.37	HS	66.82	0.23	HS	57.14	0.43	MS	68.6	0.29	HS
J07-5	0.27	45.2	0.53	MS	45.55	0.48	MS	68.22	0.32	HS	75.48	0.22	HS
J07-6	0	44.89	0.54	MS	44.29	0.49	MS	52.43	0.48	MS	70.59	0.27	HS
Shuixihong	3.13	33.82	0.65	MR	29.93	0.66	MR	39.17	0.61	MR	52	0.46	MS
Atlantic	4.95	54.68	0.43	MS	61.67	0.29	HS	68.59	0.31	HS	75	0.22	HS
Shepody	14	69.37	0.28	HS <sup>d</sup>	66.67	0.23	HS	60	0.4	MS	81.43	0.16	HS
Sepihong	1.05	35.9	0.63	MR	50.14	0.42	MS	45.91	0.54	MS	59	0.39	HS
J07-7	0	62.22	0.36	HS	43.67	0.5	MS	56.88	0.43	MS	64.76	0.33	HS
J07-2	1.34	64	0.34	HS	47.62	0.45	MS	67.14	0.33	HS	76.84	0.21	HS

<sup>a</sup> the "HR" was highly resistant.

<sup>b</sup> the "MR" was moderately resistant.

<sup>c</sup> the "MS" was moderately susceptible.

<sup>d</sup> and the "HS" highly susceptible.

in Table 4. The results of resistance evaluation for tuber black scurf in the field over 2 years showed that there were no immune or highly resistant cultivars among the 20 mate-

rials, but that most were susceptible (Table 5). Only cultivars Desiree and Longshu 6 showed moderate resistance in both years, with fewer and smaller sclerotia on tubers.

**Table 3.** Variance analysis of disease indices of potato cultivars or lines at different inoculum levels in 2009

Inoculum (g)	F-value	P-value
2	12.49	0.01
3	7.95	0.01
4	6.70	0.01
5	10.82	0.01

**Table 4.** Variance analysis of average disease index for inoculum levels of 2, 3, and 4 g in 2009 and disease index in 2010

Source of variance	SS	df	MS	F-value	P-value
Cultivars/lines	9184.84	19	340.18	4.72	0.01
Years	1182.66	1	1182.66	16.41	0.01
Error	1945.54	19	72.06		
Total variance	12313.04	39			

The correlation coefficient between the average disease index of potato tubers with inoculum levels of 2, 3, and 4 g in 2009 and disease index in 2010 was  $r = 0.661^{**}$ .

Susceptibility was relatively stable across years, with the proportion of stably susceptible cultivars reaching 75%. ‘Shepody’, ‘Atlantic’, and ‘Favorita’, the main cultivars in Inner Mongolia, showed stable susceptibility over the 2 years. Some moderately resistant and susceptible cultivars showed different results between the 2 years, changing from moderate resistance to moderate susceptibility or from moderate susceptibility to moderate resistance. ‘Shuixihong’ showed

the most obvious change, with moderate resistance in 2009 and high susceptibility in 2010. The Japanese potato lines J07-6 and J08-1 were moderately susceptible in 2009 and moderately resistant in 2010.

## Discussion

### *Development of a two-step method*

In this study, we combined the inoculation methods of laboratory tuber piece inoculum and field wheat bran inoculum and systematically studied the identification techniques of potato black scurf resistance, and proposed a two-step method for evaluating and screening potato germplasm resistant to black scurf. This simple method consists of laboratory inoculation and field inoculation. Preliminary evaluation is based on tuber piece inoculation in the laboratory, and relatively resistant cultivars are selected, then conducted resistance tests using 2–4 g of wheat bran inoculum in the field. The previous methods used for evaluating and screening potato germplasm resistant to black scurf are based on field trial (Djébali and Belhassen 2010, Naz *et al.* 2008, Scholte 1989, Simons and Gilligan 1997b), which requires a great deal of time, money, field, and labor force. And our method first used laboratory tuber piece inoculation to take preliminary evaluation for potato resistance in addition to field trial. Using tuber piece inoculation in the laboratory, some potato varieties susceptible to *R. solani* can be rapidly eliminated, and the relatively resistant varieties were used

**Table 5.** Resistance evaluation for tuber black scurf in the field in 2009 and 2010

Cultivars/lines	2009			2010			Average DI	Average RRI	Comprehensive resistance evaluation <sup>d</sup>
	DI <sup>a</sup>	RRI <sup>b</sup>	Resistance evaluation <sup>c</sup>	DI	RRI	Resistance evaluation			
Desiree	21.09	0.77	MR	29.25	0.61	MR	25.17	25.17	MR
LK99	49.35	0.47	MS	37.64	0.5	MS	43.49	43.49	MS
Zihuabai	47.07	0.5	MS	37.5	0.5	MS	42.29	42.29	MS
Kexin 1	46.22	0.51	MS	34	0.55	MS	40.11	40.11	MS
Qingshu 168	40.56	0.57	MS	43.95	0.41	MS	42.25	42.25	MS
J08-4	51.47	0.46	MS	57.75	0.23	HS	54.61	54.61	MS-HS
Longshu 7	53.59	0.43	MS	41.43	0.45	MS	47.51	47.51	MS
Longshu 3	45.35	0.52	MS	39	0.48	MS	42.18	42.18	MS
Heimeiren	29.96	0.68	MR	37.07	0.51	MS	33.52	33.52	MR-MS
Longshu 6	37.40	0.6	MR	29.07	0.61	MR	33.23	33.23	MR
J08-1	47.98	0.49	MS	22.43	0.7	MR	35.20	35.2	MS-MR
Favorita	61.76	0.34	HS	44.61	0.41	MS	53.19	53.19	HS-MS
J07-5	52.99	0.44	MS	45.51	0.39	HS	49.25	49.25	MS-HS
J07-6	47.20	0.5	MS	15.56	0.79	MR	31.38	31.38	MS-MR
Shuixihong	34.31	0.64	MR	54.76	0.27	HS	44.53	44.53	MR-HS
Atlantic	61.65	0.35	HS	52.74	0.3	HS	57.19	57.19	HS
Shepody	65.35	0.31	HS	46.5	0.38	HS	55.92	55.92	HS
Sepihong	43.98	0.53	MS	34.94	0.53	MS	39.46	39.46	MS
J07-7	54.26	0.43	MS	47.08	0.37	HS	50.67	50.67	MS-HS
J07-2	59.59	0.37	HS	52.98	0.29	HS	56.28	56.28	HS

<sup>a</sup> Average disease index of potato cultivars at 2-, 3-, and 4-g inoculum levels in 2009.

<sup>b</sup> Average relative resistance index of potato cultivars at 2-, 3-, and 4-g inoculum levels in 2009.

<sup>c</sup> Comprehensive resistance evaluation based on 2-, 3-, and 4-g inoculum levels in 2009.

<sup>d</sup> Results of resistance evaluation based on the evaluations in 2009 and 2010.

for field screening. Therefore, this simple method can save time, money, field and workload, which raising the screening efficiency of the potato varieties resistant to black scurf. In future study, the field experiment will be made in an actual infected field of the pathogen to better test and verify the two-step method. And this simple method will be used in practice to screen potato cultivars of high resistance to tuber black scurf.

#### *Tuber piece inoculation*

Tuber piece inoculation was used to evaluate resistance to black scurf caused by *R. solani*. The method that the potato pieces were inoculated with disks of *R. solani* was tried first and the infection situation was observed in laboratory. The hyphae inoculated in potato pieces expanded rapidly through the potato tissue area and generated a large number of sclerotia. The number and size of sclerotia on the tubers may be determined mainly by the nutritional components of potato. Armentrout *et al.* (1986) suggested that the surface structure of potato tubers was not the crucial factor for sclerotium formation. Some reports suggested that the formation of sclerotia was related to tuber exudation and promoted by wounded tubers, with sclerotium growth determined by the nutrients (Allington 1936, Dijst 1985, 1988). In the present study, we found that a few hyphae could penetrate the epidermis of the potato tuber and infect the interior tissue in field test, which provides the foundation for the tuber piece inoculation method described here. In the tuber piece inoculation of the present study, the inoculated hyphae grew quickly in the potato piece and caused necrosis in the potato tissue. The lesion areas on tuber pieces with *R. solani* inoculation positively correlated ( $r = 0.46^*$ ) with the disease indices of potato tubers during the 2 years of field tests. Thus, the size of lesion areas on tuber pieces positively correlated with black scurf resistance, independent of the influence of structural resistance. Compared with the main inoculum methods in field experiments that are in common use (Djébal and Belhassen 2010, Naz *et al.* 2008, Scholte 1989, Simons and Gilligan 1997b), tuber piece inoculum has the advantages of simplicity, easy control, and little environmental influence. It provides a simple and rapid method for preliminary screening potato germplasm for resistance to black scurf and verifying the results of field testing.

#### *Screening of potato germplasm for resistance to black scurf in field*

The disease indices showed a highly positive correlation ( $r = 0.66^{**}$ ) between 2009 and 2010, indicating that the results of disease resistance on potato tubers were largely consistent over 2 years and that the infection levels of the different potato cultivars or lines were relatively stable. The ANOVA of average disease index of the three inoculum levels in 2009 and disease index in 2010 showed that there were significant differences among potato materials, which indicated that the resistance of cultivars or lines was different and that it was possible to identify resistant materials in

the potato cultivars. Disease severity was different between the 2 years, with the incidence of black scurf being lower in 2010 than that in 2009.

The results also showed that the resistance to *R. solani* of the 20 potato cultivars or lines was significantly different, but none of them were completely resistant, and most of them were susceptible. The cultivar Desiree showed relatively high and stable resistance to black scurf. Bains *et al.* (2002) showed that 'Desiree' was resistant while 'Atlantic' was susceptible, consistent with our finding. Almasia *et al.* (2008) reported that the resistance gene *Snakin-1* (*SN1*) isolated from *Solanum tuberosum* cv. Desiree could be highly expressed in transgenic potato and greatly increased resistance to *R. solani*. Naz *et al.* (2008) evaluated resistance to *R. solani* in 14 potato entries and found that 'Desiree' was the most susceptible cultivar of all in greenhouse conditions.

To date, there are few available potato cultivars with high resistance to species of *Rhizoctonia* (Bains *et al.* 2002, Djébal and Belhassen 2010, Leach and Webb 1993, Naz *et al.* 2008, Olanya *et al.* 2009, Scholte 1989, Yanar *et al.* 2005). Most of the conventional cultivars are susceptible and may show severe stem canker and tuber black scurf. In Wulanchabu City, Inner Mongolia, relatively susceptible cultivars Favorita, Atlantic, and Shepody are widely planted, and the pathogen has accumulated in soil with continuous cropping for many years, leading to increasing severity of the disease.

#### *Factors that affect the evaluation of potato resistance*

The resistant differences in a few potato cultivars or lines were attributed to different inoculums levels and the methods of inoculation. Platt (1989) noted that *Rhizoctonia* disease severity is a function of host susceptibility, inoculum level and that the effects of this pathogen on potato are inconsistent. Gilligan *et al.* (1996) also observed that the results had a high degree of variability between 2 years of study. In the present study, the resistance scores of some potato materials with different soil inoculums levels showed differences. However, 2-, 3-, and 4-g inoculum levels had generally consistent proportions of resistant and susceptible, except the inoculum 5 g, which made most of cultivars or lines highly infected. Therefore, the optimum inoculation was accordingly concluded to be 2, 3, or 4 g, with 5 g inoculum not suitable for identifying potato resistance to *R. solani*. Moreover, other factors, such as genetic factors, environment, tuber maturity, cuticular structure, plant vigor, and pathogenicity that affect the expression of potato resistance may also be the reasons that affect potato resistance evaluation (Bains *et al.* 2002, Djébal and Belhassen 2010, Leach and Webb 1993, Otrysko *et al.* 1992).

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